F ENT COOPERATION TR TY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year)	
16 June 2000 (16.06.00)	
International application No. PCT/US99/23641	**Applicant's or agent's file reference** P23,495 PCT
International filing date (day/month/year) 13 October 1999 (13.10.99)	**Priority date** (day/month/year) 16 October 1998 (16.10.98)
Applicant MALISZEWSKI, Charles, R. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 May 2000 (15.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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FOR FILE 23495 USA
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SYNNESTVEDT & LECHNER
ATTEN: PJK

PCT PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
SYNNESTVEDT & LECHNER
Attn. KELLY,P
2600 ARAMARK Tower
1101 Market Street
Philadelphia, PA 19107-2950
UNITED STATES OF AMERICA

COPY

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference P23,495 PCT	Date of mailing *(day/month/year)* 17/05/2000
International application No. PCT/US 99/ 23641	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
Applicant IMMUNEX CORPORATION et al.	International filing date *(day/month/year)* 13/10/1999

ENTERED COMPUTER
7-17-00

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Nina Vercio

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, A61K 38/46, A61P 9/00, 9/10 // (A61K 38/46, 31:60)

A3

(11) International Publication Number: **WO 00/23094**

(43) International Publication Date: 27 April 2000 (27.04.00)

(21) International Application Number: PCT/US99/23641

(22) International Filing Date: 13 October 1999 (13.10.99)

(30) Priority Data:

60/104,585	16 October 1998 (16.10.98)	US
60/107,466	6 November 1998 (06.11.98)	US
60/149,010	13 August 1999 (13.08.99)	US

(71) Applicants *(for all designated States except US)*: IMMUNEX CORPORATION [US/US]; 51 University Street, Seattle, WA 98101–2936 (US). CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: MALISZEWSKI, Charles, R. [US/US]; 1014 N.W. 120th, Seattle, WA 98177 (US). GAYLE, Richard, B., III [US/US]; 17833 149th Avenue N.E., Woodinville, WA 98072 (US). MARCUS, Aaron, J. [US/US]; 41 Woods Lane, Scarsdale, NY 10583 (US).

(74) Agents: KELLY, Patrick, J.; Synnestvedt & Lechner LLP, 2600 Aramark Tower, 1101 Market Street, Philadelphia, PA 19107–2950 (US) et al.

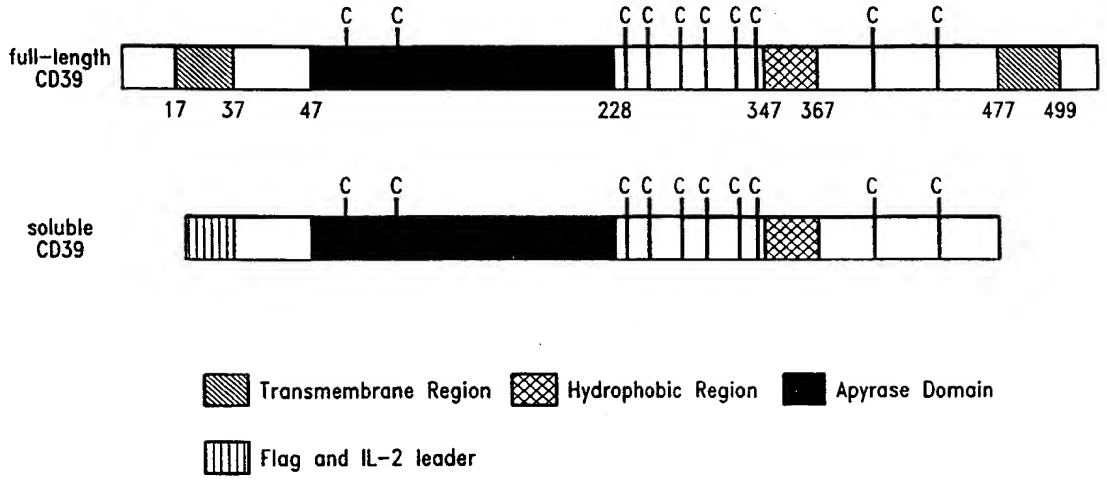
(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.
With an indication in relation to a deposited biological material furnished under Rule 13bis separately from the description.

(88) Date of publication of the international search report:
27 July 2000 (27.07.00)

(54) Title: METHODS OF INHIBITING PLATELET ACTIVATION AND RECRUITMENT



(57) Abstract

The present invention provides soluble CD39 polypeptides and compositions, and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble CD39 polypeptide.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
P23,495 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/ 23641	13/10/1999	16/10/1998
Applicant		
IMMUNEX CORPORATION et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] th text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 2

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/23641

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23641

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 A61K38/46 A61P9/00 A61P9/10 //(A61K38/46, 31:60)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GAYLE RICHARD B III ET AL: "Inhibition of platelet function by recombinant soluble ecto-ADPase/CD39." JOURNAL OF CLINICAL INVESTIGATION MAY 1, 1998, vol. 101, no. 9, 1 May 1998 (1998-05-01), pages 1851-1859, XP002136365 ISSN: 0021-9738 cited in the application the whole document --- -/--	1-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 April 2000	17/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Niemann, F

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 30532 A (SANDOZ LTD ;NEW ENGLAND DEACONESS HOSPITAL (US); BACH FRITZ H (US)) 3 October 1996 (1996-10-03) page 2, line 6 -page 4, line 35 page 6, line 2 - line 15 page 11, line 12 - line 36 page 15, line 12 -page 16, line 27 page 20, line 25 - line 32 page 28, line 8 -page 29, line 32; claims 14,34-41; figure 14	1,2,9, 12-20
A	CHADWICK B P ET AL: "The CD39-like gene family: identification of three new human members (CD39L2, CD39L3, and CD39L4), their murine homologues, and a member of the gene family from Drosophila melanogaster" GENOMICS,US,ACADEMIC PRESS, SAN DIEGO, vol. 50, no. 3, 15 June 1998 (1998-06-15), pages 357-367, XP002117226 ISSN: 0888-7543 the whole document	1-4,6-8
A	MARCUS AARON J ET AL: "The endothelial cell ecto-ADPase responsible for inhibition of platelet function is CD39." JOURNAL OF CLINICAL INVESTIGATION 1997, vol. 99, no. 6, 1997, pages 1351-1360, XP002136366 ISSN: 0021-9738 the whole document	1,17,20
A	CULLEN B. R.: "expression of a cloned human interleukin-2 cdna is emhanced by the substitution of a heterologous mrna leader region" DNA, vol. 7, no. 9, 1988, pages 645-650, XP000892169 the whole document	3-8,10, 11
A	WO 96 32471 A (UNIV SHERBROOKE ;BEAUDOIN ADRIEN R (CA); SEVIGNY JEAN (CA)) 17 October 1996 (1996-10-17) claims 1,14-16	1-20
A	EP 0 416 673 A (CIGB) 13 March 1991 (1991-03-13) page 3, line 1 - line 51 sequence no 7 abstract; claims 1,2	3-5

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/23641

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 073 627 A (CURTIS BENSON M ET AL) 17 December 1991 (1991-12-17) cited in the application column 2, line 21 - line 27 column 6, line 31 -column 7, line 7; claims 1-3 -----	5

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/23641

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9630532 A	03-10-1996	AU 5147996 A	16-10-1996
		CA 2216445 A	03-10-1996
		EP 0815252 A	07-01-1998
		JP 11503905 T	06-04-1999
WO 9632471 A	17-10-1996	AU 5265296 A	30-10-1996
		US 5798241 A	25-08-1998
EP 0416673 A	13-03-1991	CU 22222 B	28-03-1994
		AT 130370 T	15-12-1995
		DE 69023580 D	21-12-1995
		DE 69023580 T	11-04-1996
		ES 2081913 T	16-03-1996
		JP 4158797 A	01-06-1992
US 5073627 A	17-12-1991	IE 64202 B	12-07-1995
		MX 9203426 A	01-07-1992
		US 5108910 A	28-04-1992
		AT 103932 T	15-04-1994
		AU 632372 B	24-12-1992
		AU 6424090 A	03-04-1991
		DD 297188 A	02-01-1992
		DE 69007975 D	11-05-1994
		DE 69007975 T	21-07-1994
		DK 489116 T	02-05-1994
		EP 0489116 A	10-06-1992
		ES 2055445 T	16-08-1994
		JP 5500806 T	18-02-1993
		NO 301888 B	22-12-1997
		WO 9102754 A	07-03-1991

Immunoprecipitation of HUVEC detergent lysates with anti-CD39 mAb resulted in complete capture of cell-associated ADPase activity, suggesting that CD39 is the only ecto-ADPase on endothelial cells (Marcus et al., *J. Clin. Invest.* 99:1351, 1997). In the same study, COS cell transfectants expressing recombinant CD39 at the cell surface totally inhibited ADP-induced platelet aggregation. Thus, CD39 plays a prominent role in thromboregulation (*see also,* Gayle et al., *J. Clin. Invest.*, 101:1851, 1998).

Excessive platelet activation (i.e., stimulation by an agonist) and recruitment, leading to platelet aggregation and vessel occlusion at sites of vascular injury in the coronary, carotid, and peripheral arteries, presents a major therapeutic challenge in cardiovascular medicine. Excessive platelet activation and recruitment is a contributing factor in clinical disorders including stroke, unstable angina, myocardial infarction, and restenosis following percutaneous coronary intervention including angioplasty, atherectomy, stent placement, and bypass surgery.

Glycoprotein IIb/IIIa antagonists, such as the monoclonal antibody marketed as ReoPro® (Centocor Inc.), are presently under development for the inhibition of platelet aggregation in patients undergoing percutaneous coronary intervention, and in patients with acute coronary syndromes such as unstable angina and myocardial infarction. The activation of glycoprotein IIb/IIIa receptors, however, is a late event in the cascade that leads to platelet aggregation.

There is a great need to identify additional therapeutic strategies and compositions for the pharmacological neutralization of platelet reactivity (activation, recruitment, aggregation). In particular, there is a need to identify compounds and compositions which target early portions of coagulation pathways such as the ADP-dependent activation and recruitment of platelets. There is, in fact, an urgent need to identify new strategies and compositions for the treatment of stroke, which is the third leading cause of death in the United States. In the case of stroke, an advantageous therapeutic agent will reduce intravascular thrombus burden and accompanying neurological defects without increasing intracerebral hemorrhage.

SUMMARY OF THE INVENTION

Soluble forms of CD39 having apyrase activity constitute a novel approach to the prevention and/or treatment of disease. The present invention provides soluble CD39 polypeptides and nucleic acids, compositions comprising a pharmaceutically acceptable carrier and a soluble CD39 polypeptide, and methods of making and using soluble CD39 polypeptides having apyrase activity. The effectiveness of soluble CD39 polypeptides have been demonstrated in vitro, ex vivo, and in vivo.

The invention is directed to soluble CD39 polypeptides selected from the group consisting of: (a) polypeptides having an amino acid sequence as set forth in Figure 1 (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478; (b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; (c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and (d) fusion polypeptides comprising the polypeptides of (a), (b),

CLAIMS

We claim:

1. A method for inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in Figure 1 (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity;

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides have apyrase activity.

2. The method of claim 1 wherein the polypeptide is selected from the group consisting of:

(a) polypeptides having a sequence consisting of amino acids 38-476 or 39-476 of SEQ ID NO:2;

(b) variant polypeptides that are at least 70% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(c) variant polypeptides that are at least 80% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(d) variant polypeptides that are at least 90% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(e) variant polypeptides that are at least 95% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(f) variant polypeptides that are at least 98% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity; and

(g) variant polypeptides that are at least 99% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity.

3. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a polypeptide having the structure X-Y wherein Y is the soluble CD39 polypeptide of claim 1 and X is selected from the group consisting of an Ala residue and peptides capable of adopting a stable secondary structure.

4. The method of claim 3 wherein X is a peptide fragment from the amino terminal portion of mature IL-2, CD39-L2, CD39-L3, or CD39-L4.

5. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a polypeptide having the structure A-B-C wherein A is 0-20 amino acids from the amino terminal portion of mature IL-2, B is a linker of 0-15 amino acids, and C is the soluble CD39 polypeptide of claim 1.

6. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide selected from the group consisting of:

(a) SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, or amino acids 21-463 of SEQ ID NO:30;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity;

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides have apyrase activity.

7. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a polypeptide selected from the group consisting of:

(a) variant polypeptides that are at least 70% identical in amino acid sequence to the soluble CD39 polypeptide of claim 6, wherein said variant polypeptides have apyrase activity;

(d) variant polypeptides that are at least 80% identical in amino acid sequence to the soluble CD39 polypeptide of claim 6, wherein said variant polypeptides have apyrase activity;

(e) variant polypeptides that are at least 90% identical in amino acid sequence to the soluble CD39 polypeptide of claim 6, wherein said variant polypeptides have apyrase activity;

(f) variant polypeptides that are at least 95% identical in amino acid sequence to the soluble CD39 polypeptide of claim 6, wherein said variant polypeptides have apyrase activity;

(g) variant polypeptides that are at least 98% identical in amino acid sequence to the soluble CD39 polypeptide of claim 6, wherein said variant polypeptides have apyrase activity; and

(h) variant polypeptides that are at least 99% identical in amino acid sequence to the soluble CD39 polypeptide of claim 6, wherein said variant polypeptides have apyrase activity.

8. The method of claim 6 wherein the soluble CD39 polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, and amino acids 21-463 of SEQ ID NO:30.

9. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide that has been produced by culturing a recombinant cell that encodes a soluble CD39 polypeptide according to claim 1 under conditions permitting expression of the CD39 polypeptide, and recovering the expressed CD39 polypeptide.

10. The method of claim 9 wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:5;

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:5;

(c) DNA sequences that hybridize to SEQ ID NO:5 under moderately stringent conditions;

(d) DNA sequences that are at least 70% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(e) DNA sequences that are at least 80% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(f) DNA sequences that are at least 90% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(g) DNA sequences that are at least 95% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(h) DNA sequences that are at least 98% identical in sequence to SEQ ID NO:5 or to a fragment thereof; and

(i) DNA sequences that are at least 99% identical in sequence to SEQ ID NO:5 or to a fragment thereof.

11. The method of claim 9 wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:7;

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:7;

(c) DNA sequences which hybridize to SEQ ID NO:7 under moderately stringent conditions;

(d) DNA sequences that are at least 70% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(e) DNA sequences that are at least 80% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(f) DNA sequences that are at least 90% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(g) DNA sequences that are at least 95% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(h) DNA sequences that are at least 98% identical in sequence to SEQ ID NO:7 or to a fragment thereof; and

(i) DNA sequences that are at least 99% identical in sequence to SEQ ID NO:7 or to a fragment thereof.

12. The method of one of claims 1-11 wherein the soluble CD39 polypeptide is administered in a composition comprising a pharmaceutically acceptable carrier.

13. The method of one of claims 1-11 wherein the soluble CD39 polypeptide is administered in combination with at least one other antithrombotic or antiplatelet composition.

14. The method of claim 13 wherein the soluble CD39 polypeptide is administered in combination with aspirin.

15. The method of one of claims 1-11 wherein the soluble CD39 polypeptide is administered parenterally.

16. The method of claim 15 wherein the solubleCD39 polypeptide is administered intravenously.

17. The method of one of claims 1-11 wherein the mammal is suffering from unstable angina, myocardial infarction, stroke, coronary artery disease or injury, myocardial infarction, atherosclerosis, peripheral vascular occlusion, preeclampsia, embolism, a platelet-associated ischemic disorder including lung ischemia, coronary ischemia, and cerebral ischemia, a thrombotic disorder including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombosis and coagulopathy associated with exposure to a foreign or injured tissue surface, deep venous thrombosis (DVT), pulmonary embolism (PE), transient ischemic attack (TIAs), or another related condition where vascular occlusion is the common underlying feature.

18. The method of one of claims 1-11 wherein the soluble CD39 is administered to prevent thrombus formation or reformation, occlusion, reocclusion, stenosis, or restenosis of blood vessels, or stroke.

19. The method of one of claims 1-11 wherein the soluble CD39 is administered in conjunction with angioplasty, carotid endarterectomy, anastomosis of vascular graft, atherectomy, stent placement, placement of a chronic cardiovascular device such as an in-dwelling catheter or prosthetic valve or vessel, or bypass surgery.

20. A method for degrading nucleoside tri- and/or di- phosphates in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in Figure 1 (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity;

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides have apyrase activity.

SEQUENCE LISTING

<110> Maliszewski, Charles R.
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<120> Methods of Inhibiting Platelet Activation and Recruitment

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<150> US 60/104,585
<151> 1998-10-16

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                115                 120                 125

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cag gtt gca agt aat gaa att ctc agg gac cca tgc ttt cat cct gga   924
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Thr Tyr Ile Leu Ser Leu Leu Leu Gln Gly Tyr His Phe Thr Ala Asp
            420                 425                 430

Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala
        435                 440                 445

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Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly
                405                 410                 415

Thr Tyr Ile Leu Ser Leu Leu Leu Gln Gly Tyr His Phe Thr Ala Asp
            420                 425                 430

Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala
        435                 440                 445

Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile Pro Ala
    450                 455                 460

Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr
465                 470                 475


<210> 5
<211> 1365
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:  Fusion
      construct of human CD39

<220>
<221> CDS
<222> (1)..(1362)

<400> 5
gca cct act tca agt tct aca aag aaa aca cag cta act agt tca acc   48
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Thr Ser Ser Thr
  1               5                  10                  15

cag aac aaa gca ttg cca gaa aac gtt aag tat ggg att gtg ctg gat   96
Gln Asn Lys Ala Leu Pro Glu Asn Val Lys Tyr Gly Ile Val Leu Asp
            20                  25                  30

gcg ggt tct tct cac aca agt tta tac atc tat aag tgg cca gca gaa   144
Ala Gly Ser Ser His Thr Ser Leu Tyr Ile Tyr Lys Trp Pro Ala Glu
        35                  40                  45

aag gag aat gac aca ggc gtg gtg cat caa gta gaa gaa tgc agg gtt   192
Lys Glu Asn Asp Thr Gly Val Val His Gln Val Glu Glu Cys Arg Val
    50                  55                  60

aaa ggt cct gga atc tca aaa ttt gtt cag aaa gta aat gaa ata ggc   240
Lys Gly Pro Gly Ile Ser Lys Phe Val Gln Lys Val Asn Glu Ile Gly
65                  70                  75                  80

att tac ctg act gat tgc atg gaa aga gct agg gaa gtg att cca agg   288
Ile Tyr Leu Thr Asp Cys Met Glu Arg Ala Arg Glu Val Ile Pro Arg
                85                  90                  95

tcc cag cac caa gag aca ccc gtt tac ctg gga gcc acg gca ggc atg   336
```

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Ser Gln His Gln Glu Thr Pro Val Tyr Leu Gly Ala Thr Ala Gly Met
            100                 105                 110

cgg ttg ctc agg atg gaa agt gaa gag ttg gca gac agg gtt ctg gat   384
Arg Leu Leu Arg Met Glu Ser Glu Glu Leu Ala Asp Arg Val Leu Asp
        115                 120                 125

gtg gtg gag agg agc ctc agc aac tac ccc ttt gac ttc cag ggt gcc   432
Val Val Glu Arg Ser Leu Ser Asn Tyr Pro Phe Asp Phe Gln Gly Ala
    130                 135                 140

agg atc att act ggc caa gag gaa ggt gcc tat ggc tgg att act atc   480
Arg Ile Ile Thr Gly Gln Glu Glu Gly Ala Tyr Gly Trp Ile Thr Ile
145                 150                 155                 160

aac tat ctg ctg ggc aaa ttc agt cag aaa aca agg tgg ttc agc ata   528
Asn Tyr Leu Leu Gly Lys Phe Ser Gln Lys Thr Arg Trp Phe Ser Ile
                165                 170                 175

gtc cca tat gaa acc aat aat cag gaa acc ttt gga gct ttg gac ctt   576
Val Pro Tyr Glu Thr Asn Asn Gln Glu Thr Phe Gly Ala Leu Asp Leu
            180                 185                 190

ggg gga gcc tct aca caa gtc act ttt gta ccc caa aac cag act atc   624
Gly Gly Ala Ser Thr Gln Val Thr Phe Val Pro Gln Asn Gln Thr Ile
        195                 200                 205

gag tcc cca gat aat gct ctg caa ttt cgc ctc tat ggc aag gac tac   672
Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg Leu Tyr Gly Lys Asp Tyr
    210                 215                 220

aat gtc tac aca cat agc ttc ttg tgc tat ggg aag gat cag gca ctc   720
Asn Val Tyr Thr His Ser Phe Leu Cys Tyr Gly Lys Asp Gln Ala Leu
225                 230                 235                 240

tgg cag aaa ctg gcc aag gac att cag gtt gca agt aat gaa att ctc   768
Trp Gln Lys Leu Ala Lys Asp Ile Gln Val Ala Ser Asn Glu Ile Leu
                245                 250                 255

agg gac cca tgc ttt cat cct gga tat aag aag gta gtg aac gta agt   816
Arg Asp Pro Cys Phe His Pro Gly Tyr Lys Lys Val Val Asn Val Ser
            260                 265                 270

gac ctt tac aag acc ccc tgc acc aag aga ttt gag atg act ctt cca   864
Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg Phe Glu Met Thr Leu Pro
        275                 280                 285

ttc cag cag ttt gaa atc cag ggt att gga aac tat caa caa tgc cat   912
Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly Asn Tyr Gln Gln Cys His
    290                 295                 300

caa agc atc ctg gag ctc ttc aac acc agt tac tgc cct tac tcc cag   960
Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser Tyr Cys Pro Tyr Ser Gln
305                 310                 315                 320

tgt gcc ttc aat ggg att ttc ttg cca cca ctc cag ggg gat ttt ggg   1008
Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro Leu Gln Gly Asp Phe Gly
                325                 330                 335
```

```
gca ttt tca gct ttt tac ttt gtg atg aag ttt tta aac ttg aca tca   1056
Ala Phe Ser Ala Phe Tyr Phe Val Met Lys Phe Leu Asn Leu Thr Ser
            340                 345                 350

gag aaa gtc tct cag gaa aag gtg act gag atg atg aaa aag ttc tgt   1104
Glu Lys Val Ser Gln Glu Lys Val Thr Glu Met Met Lys Lys Phe Cys
        355                 360                 365

gct cag cct tgg gag gag ata aaa aca tct tac gct gga gta aag gag   1152
Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser Tyr Ala Gly Val Lys Glu
    370                 375                 380

aag tac ctg agt gaa tac tgc ttt tct ggt acc tac att ctc tcc ctc   1200
Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly Thr Tyr Ile Leu Ser Leu
385                 390                 395                 400

ctt ctg caa ggc tat cat ttc aca gct gat tcc tgg gag cac atc cat   1248
Leu Leu Gln Gly Tyr His Phe Thr Ala Asp Ser Trp Glu His Ile His
                405                 410                 415

ttc att ggc aag atc cag ggc agc gac gcc ggc tgg act ttg ggc tac   1296
Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala Gly Trp Thr Leu Gly Tyr
            420                 425                 430

atg ctg aac ctg acc aac atg atc cca gct gag caa cca ttg tcc aca   1344
Met Leu Asn Leu Thr Asn Met Ile Pro Ala Glu Gln Pro Leu Ser Thr
        435                 440                 445

cct ctc tcc cac tcc acc taa                                      1365
Pro Leu Ser His Ser Thr
    450
```

<210> 6
<211> 454
<212> PRT
<213> Artificial Sequence

<400> 6

```
Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Thr Ser Ser Thr
  1               5                  10                  15

Gln Asn Lys Ala Leu Pro Glu Asn Val Lys Tyr Gly Ile Val Leu Asp
            20                  25                  30

Ala Gly Ser Ser His Thr Ser Leu Tyr Ile Tyr Lys Trp Pro Ala Glu
        35                  40                  45

Lys Glu Asn Asp Thr Gly Val Val His Gln Val Glu Glu Cys Arg Val
    50                  55                  60

Lys Gly Pro Gly Ile Ser Lys Phe Val Gln Lys Val Asn Glu Ile Gly
65                  70                  75                  80

Ile Tyr Leu Thr Asp Cys Met Glu Arg Ala Arg Glu Val Ile Pro Arg
                85                  90                  95
```

```
Ser Gln His Gln Glu Thr Pro Val Tyr Leu Gly Ala Thr Ala Gly Met
            100                 105                 110

Arg Leu Leu Arg Met Glu Ser Glu Glu Leu Ala Asp Arg Val Leu Asp
        115                 120                 125

Val Val Glu Arg Ser Leu Ser Asn Tyr Pro Phe Asp Phe Gln Gly Ala
    130                 135                 140

Arg Ile Ile Thr Gly Gln Glu Glu Gly Ala Tyr Gly Trp Ile Thr Ile
145                 150                 155                 160

Asn Tyr Leu Leu Gly Lys Phe Ser Gln Lys Thr Arg Trp Phe Ser Ile
                165                 170                 175

Val Pro Tyr Glu Thr Asn Asn Gln Glu Thr Phe Gly Ala Leu Asp Leu
            180                 185                 190

Gly Gly Ala Ser Thr Gln Val Thr Phe Val Pro Gln Asn Gln Thr Ile
        195                 200                 205

Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg Leu Tyr Gly Lys Asp Tyr
    210                 215                 220

Asn Val Tyr Thr His Ser Phe Leu Cys Tyr Gly Lys Asp Gln Ala Leu
225                 230                 235                 240

Trp Gln Lys Leu Ala Lys Asp Ile Gln Val Ala Ser Asn Glu Ile Leu
                245                 250                 255

Arg Asp Pro Cys Phe His Pro Gly Tyr Lys Lys Val Val Asn Val Ser
            260                 265                 270

Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg Phe Glu Met Thr Leu Pro
        275                 280                 285

Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly Asn Tyr Gln Gln Cys His
    290                 295                 300

Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser Tyr Cys Pro Tyr Ser Gln
305                 310                 315                 320

Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro Leu Gln Gly Asp Phe Gly
                325                 330                 335

Ala Phe Ser Ala Phe Tyr Phe Val Met Lys Phe Leu Asn Leu Thr Ser
            340                 345                 350

Glu Lys Val Ser Gln Glu Lys Val Thr Glu Met Met Lys Lys Phe Cys
        355                 360                 365

Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser Tyr Ala Gly Val Lys Glu
    370                 375                 380

Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly Thr Tyr Ile Leu Ser Leu
385                 390                 395                 400
```

```
Leu Leu Gln Gly Tyr His Phe Thr Ala Asp Ser Trp Glu His Ile His
                405                 410                 415

Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala Gly Trp Thr Leu Gly Tyr
            420                 425                 430

Met Leu Asn Leu Thr Asn Met Ile Pro Ala Glu Gln Pro Leu Ser Thr
        435                 440                 445

Pro Leu Ser His Ser Thr
    450
```

<210> 7
<211> 1437
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<220>
<221> CDS
<222> (1)..(1434)

<400> 7

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atg gcc ctg tgg atc gac agg atg caa ctc ctg tct tgc att gca cta   48
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1               5                  10                  15

agt ctt gca ctt gtc aca aac agt gca cct act tca agt tct aca aag   96
Ser Leu Ala Leu Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys
            20                  25                  30

aaa aca cag cta act agt tca acc cag aac aaa gca ttg cca gaa aac   144
Lys Thr Gln Leu Thr Ser Ser Thr Gln Asn Lys Ala Leu Pro Glu Asn
        35                  40                  45

gtt aag tat ggg att gtg ctg gat gcg ggt tct tct cac aca agt tta   192
Val Lys Tyr Gly Ile Val Leu Asp Ala Gly Ser Ser His Thr Ser Leu
    50                  55                  60

tac atc tat aag tgg cca gca gaa aag gag aat gac aca ggc gtg gtg   240
Tyr Ile Tyr Lys Trp Pro Ala Glu Lys Glu Asn Asp Thr Gly Val Val
65                  70                  75                  80

cat caa gta gaa gaa tgc agg gtt aaa ggt cct gga atc tca aaa ttt   288
His Gln Val Glu Glu Cys Arg Val Lys Gly Pro Gly Ile Ser Lys Phe
                85                  90                  95

gtt cag aaa gta aat gaa ata ggc att tac ctg act gat tgc atg gaa   336
Val Gln Lys Val Asn Glu Ile Gly Ile Tyr Leu Thr Asp Cys Met Glu
            100                 105                 110

aga gct agg gaa gtg att cca agg tcc cag cac caa gag aca ccc gtt   384
Arg Ala Arg Glu Val Ile Pro Arg Ser Gln His Gln Glu Thr Pro Val
        115                 120                 125
```

```
tac ctg gga gcc acg gca ggc atg cgg ttg ctc agg atg gaa agt gaa   432
Tyr Leu Gly Ala Thr Ala Gly Met Arg Leu Leu Arg Met Glu Ser Glu
    130                 135                 140

gag ttg gca gac agg gtt ctg gat gtg gtg gag agg agc ctc agc aac   480
Glu Leu Ala Asp Arg Val Leu Asp Val Val Glu Arg Ser Leu Ser Asn
145                 150                 155                 160

tac ccc ttt gac ttc cag ggt gcc agg atc att act ggc caa gag gaa   528
Tyr Pro Phe Asp Phe Gln Gly Ala Arg Ile Ile Thr Gly Gln Glu Glu
                165                 170                 175

ggt gcc tat ggc tgg att act atc aac tat ctg ctg ggc aaa ttc agt   576
Gly Ala Tyr Gly Trp Ile Thr Ile Asn Tyr Leu Leu Gly Lys Phe Ser
            180                 185                 190

cag aaa aca agg tgg ttc agc ata gtc cca tat gaa acc aat aat cag   624
Gln Lys Thr Arg Trp Phe Ser Ile Val Pro Tyr Glu Thr Asn Asn Gln
        195                 200                 205

gaa acc ttt gga gct ttg gac ctt ggg gga gcc tct aca caa gtc act   672
Glu Thr Phe Gly Ala Leu Asp Leu Gly Gly Ala Ser Thr Gln Val Thr
    210                 215                 220

ttt gta ccc caa aac cag act atc gag tcc cca gat aat gct ctg caa   720
Phe Val Pro Gln Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu Gln
225                 230                 235                 240

ttt cgc ctc tat ggc aag gac tac aat gtc tac aca cat agc ttc ttg   768
Phe Arg Leu Tyr Gly Lys Asp Tyr Asn Val Tyr Thr His Ser Phe Leu
                245                 250                 255

tgc tat ggg aag gat cag gca ctc tgg cag aaa ctg gcc aag gac att   816
Cys Tyr Gly Lys Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp Ile
            260                 265                 270

cag gtt gca agt aat gaa att ctc agg gac cca tgc ttt cat cct gga   864
Gln Val Ala Ser Asn Glu Ile Leu Arg Asp Pro Cys Phe His Pro Gly
        275                 280                 285

tat aag aag gta gtg aac gta agt gac ctt tac aag acc ccc tgc acc   912
Tyr Lys Lys Val Val Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys Thr
    290                 295                 300

aag aga ttt gag atg act ctt cca ttc cag cag ttt gaa atc cag ggt   960
Lys Arg Phe Glu Met Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln Gly
305                 310                 315                 320

att gga aac tat caa caa tgc cat caa agc atc ctg gag ctc ttc aac  1008
Ile Gly Asn Tyr Gln Gln Cys His Gln Ser Ile Leu Glu Leu Phe Asn
                325                 330                 335

acc agt tac tgc cct tac tcc cag tgt gcc ttc aat ggg att ttc ttg  1056
Thr Ser Tyr Cys Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe Leu
            340                 345                 350

cca cca ctc cag ggg gat ttt ggg gca ttt tca gct ttt tac ttt gtg  1104
```

```
Pro Pro Leu Gln Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe Val
        355                 360                 365

atg aag ttt tta aac ttg aca tca gag aaa gtc tct cag gaa aag gtg   1152
Met Lys Phe Leu Asn Leu Thr Ser Glu Lys Val Ser Gln Glu Lys Val
    370                 375                 380

act gag atg atg aaa aag ttc tgt gct cag cct tgg gag gag ata aaa   1200
Thr Glu Met Met Lys Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile Lys
385                 390                 395                 400

aca tct tac gct gga gta aag gag aag tac ctg agt gaa tac tgc ttt   1248
Thr Ser Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe
                405                 410                 415

tct ggt acc tac att ctc tcc ctc ctt ctg caa ggc tat cat ttc aca   1296
Ser Gly Thr Tyr Ile Leu Ser Leu Leu Leu Gln Gly Tyr His Phe Thr
            420                 425                 430

gct gat tcc tgg gag cac atc cat ttc att ggc aag atc cag ggc agc   1344
Ala Asp Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser
        435                 440                 445

gac gcc ggc tgg act ttg ggc tac atg ctg aac ctg acc aac atg atc   1392
Asp Ala Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile
    450                 455                 460

cca gct gag caa cca ttg tcc aca cct ctc tcc cac tcc acc taa       1437
Pro Ala Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr
465                 470                 475


<210> 8
<211> 478
<212> PRT
<213> Artificial Sequence

<400> 8
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1               5                  10                  15

Ser Leu Ala Leu Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys
            20                  25                  30

Lys Thr Gln Leu Thr Ser Ser Thr Gln Asn Lys Ala Leu Pro Glu Asn
        35                  40                  45

Val Lys Tyr Gly Ile Val Leu Asp Ala Gly Ser Ser His Thr Ser Leu
    50                  55                  60

Tyr Ile Tyr Lys Trp Pro Ala Glu Lys Glu Asn Asp Thr Gly Val Val
65                  70                  75                  80

His Gln Val Glu Glu Cys Arg Val Lys Gly Pro Gly Ile Ser Lys Phe
                85                  90                  95

Val Gln Lys Val Asn Glu Ile Gly Ile Tyr Leu Thr Asp Cys Met Glu
            100                 105                 110
```

```
Arg Ala Arg Glu Val Ile Pro Arg Ser Gln His Gln Glu Thr Pro Val
        115                 120                 125

Tyr Leu Gly Ala Thr Ala Gly Met Arg Leu Leu Arg Met Glu Ser Glu
    130                 135                 140

Glu Leu Ala Asp Arg Val Leu Asp Val Val Glu Arg Ser Leu Ser Asn
145                 150                 155                 160

Tyr Pro Phe Asp Phe Gln Gly Ala Arg Ile Ile Thr Gly Gln Glu Glu
                165                 170                 175

Gly Ala Tyr Gly Trp Ile Thr Ile Asn Tyr Leu Leu Gly Lys Phe Ser
            180                 185                 190

Gln Lys Thr Arg Trp Phe Ser Ile Val Pro Tyr Glu Thr Asn Asn Gln
        195                 200                 205

Glu Thr Phe Gly Ala Leu Asp Leu Gly Gly Ala Ser Thr Gln Val Thr
    210                 215                 220

Phe Val Pro Gln Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu Gln
225                 230                 235                 240

Phe Arg Leu Tyr Gly Lys Asp Tyr Asn Val Tyr Thr His Ser Phe Leu
                245                 250                 255

Cys Tyr Gly Lys Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp Ile
            260                 265                 270

Gln Val Ala Ser Asn Glu Ile Leu Arg Asp Pro Cys Phe His Pro Gly
        275                 280                 285

Tyr Lys Lys Val Val Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys Thr
    290                 295                 300

Lys Arg Phe Glu Met Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln Gly
305                 310                 315                 320

Ile Gly Asn Tyr Gln Gln Cys His Gln Ser Ile Leu Glu Leu Phe Asn
                325                 330                 335

Thr Ser Tyr Cys Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe Leu
            340                 345                 350

Pro Pro Leu Gln Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe Val
        355                 360                 365

Met Lys Phe Leu Asn Leu Thr Ser Glu Lys Val Ser Gln Glu Lys Val
    370                 375                 380

Thr Glu Met Met Lys Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile Lys
385                 390                 395                 400

Thr Ser Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe
                405                 410                 415
```

Ser Gly Thr Tyr Ile Leu Ser Leu Leu Leu Gln Gly Tyr His Phe Thr
420 425 430

Ala Asp Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser
435 440 445

Asp Ala Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile
450 455 460

Pro Ala Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr
465 470 475

<210> 9
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic signal sequence

<400> 9
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
1 5 10 15

Ser Leu Ala Leu Val Thr Asn Ser
20

<210> 10
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 10
Asp Tyr Lys Asp Asp Asp Asp Lys
1 5

<210> 11
<211> 43
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 11
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
1 5 10 15

Ser Leu Ala Leu Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys
20 25 30

Lys Thr Gln Leu Thr Ser Ser Thr Gln Asn Lys
35 40

<210> 12
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 12
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
1 5 10 15

Ser Leu Ala Leu Val Thr Asn Ser Ala Thr Gln Asn Lys
20 25

<210> 13
<211> 31
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 13
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
1 5 10 15

Ser Leu Ala Leu Val Thr Asn Ser Ala Ser Ser Thr Gln Asn Lys
20 25 30

<210> 14
<211> 87
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 14
ccggctggac tttgggctac atgctgaacc tgaccaacat gatcccagct gagcaaccat 60

tgtccacacc tctctcccac gagcccc 87

<210> 15
<211> 87

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 15
gatcggggct cgtgggagag aggtgtggac aatggttgct cagctgggat catgttggtc 60

aggttcagca tgtagcccaa agtccag 87

<210> 16
<211> 740
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (42)..(737)

<400> 16
cggtaccgct agcgtcgaca ggcctaggat atcgatacgt a gag ccc aga tct tgt 56
Glu Pro Arg Ser Cys
1 5

gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa gcc gag ggc 104
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Ala Glu Gly
10 15 20

gcg ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg 152
Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
25 30 35

atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac 200
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
40 45 50

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg 248
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
55 60 65

cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac 296
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
70 75 80 85

cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc 344
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
90 95 100

aag gac tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atg 392
Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Met
105 110 115

cag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg 440
Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
120 125 130

```
tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc   488
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
    135                 140                 145

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agg cac atc gcc gtg gag   536
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg His Ile Ala Val Glu
150                 155                 160                 165

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc   584
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                170                 175                 180

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg   632
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            185                 190                 195

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg   680
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        200                 205                 210

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct   728
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    215                 220                 225

ccg ggt aaa tga                                                   740
Pro Gly Lys
230
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<210> 17
<211> 232
<212> PRT
<213> Homo sapiens

<400> 17

```
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
  1               5                  10                  15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110
```

Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
115 120 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
130 135 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145 150 155 160

His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
165 170 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
180 185 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
195 200 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
210 215 220

Ser Leu Ser Leu Ser Pro Gly Lys
225 230

<210> 18
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 18
ctttccatcc tgagcaac 18

<210> 19
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 19
aaaaaactag tcagaacaaa gctttgccag aaaacg 36

<210> 20
<211> 24
<212> PRT
<213> Mus sp.

<400> 20

Met Phe His Val Ser Phe Arg Tyr Ile Phe Gly Ile Pro Pro Leu Ile
1 5 10 15

Leu Val Leu Leu Pro Val Thr Ser
20

<210> 21
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 21
ctagttctgg agactacaaa gatgacgatg acaaaaccca gaacaa 46

<210> 22
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 22
agctttgttc tgggttttgt catcgtcatc tttgtagtct ccagaa 46

<210> 23
<211> 89
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 23
ccggctggac tttgggctac atgctgaacc tgaccaacat gatcccagct gagcaaccat 60
tgtccacacc tctctcccac tccacctaa 89

<210> 24
<211> 89
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 24

ggccttaggt ggagtgggag agaggtgtgg acaatggttg ctcagctggg atcatgttgg 60

tcaggttcag catgtagccc aaagtccag 89

<210> 25
<211> 1464
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (1)..(1461)

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 25

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atg gcc ctg tgg atc gac agg atg caa ctc ctg tct tgc att gca cta   48
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1               5                  10                  15

agt ctt gca ctt gtc aca aac agt gca cct act tca agt tct aca aag   96
Ser Leu Ala Leu Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys
             20                  25                  30

aaa aca cag cta act agt tca gga gac tac aaa gat gac gat gac aaa   144
Lys Thr Gln Leu Thr Ser Ser Gly Asp Tyr Lys Asp Asp Asp Asp Lys
         35                  40                  45

acc cag aac aaa gca ttg cca gaa aac gtt aag tat ggg att gtg ctg   192
Thr Gln Asn Lys Ala Leu Pro Glu Asn Val Lys Tyr Gly Ile Val Leu
     50                  55                  60

gat gcg ggt tct tct cac aca agt tta tac atc tat aag tgg cca gca   240
Asp Ala Gly Ser Ser His Thr Ser Leu Tyr Ile Tyr Lys Trp Pro Ala
 65                  70                  75                  80

gaa aag gag aat gac aca ggc gtg gtg cat caa gta gaa gaa tgc agg   288
Glu Lys Glu Asn Asp Thr Gly Val Val His Gln Val Glu Glu Cys Arg
                 85                  90                  95

gtt aaa ggt cct gga atc tca aaa ttt gtt cag aaa gta aat gaa ata   336
Val Lys Gly Pro Gly Ile Ser Lys Phe Val Gln Lys Val Asn Glu Ile
            100                 105                 110

ggc att tac ctg act gat tgc atg gaa aga gct agg gaa gtg att cca   384
Gly Ile Tyr Leu Thr Asp Cys Met Glu Arg Ala Arg Glu Val Ile Pro
        115                 120                 125

agg tcc cag cac caa gag aca ccc gtt tac ctg gga gcc acg gca ggc   432
Arg Ser Gln His Gln Glu Thr Pro Val Tyr Leu Gly Ala Thr Ala Gly
    130                 135                 140

atg cgg ttg ctc agg atg gaa agt gaa gag ttg gca gac agg gtt ctg   480
Met Arg Leu Leu Arg Met Glu Ser Glu Glu Leu Ala Asp Arg Val Leu
145                 150                 155                 160
```

```
gat gtg gtg gag agg agc ctc agc aac tac ccc ttt gac ttc cag ggt   528
Asp Val Val Glu Arg Ser Leu Ser Asn Tyr Pro Phe Asp Phe Gln Gly
                165                 170                 175

gcc agg atc att act ggc caa gag gaa ggt gcc tat ggc tgg att act   576
Ala Arg Ile Ile Thr Gly Gln Glu Glu Gly Ala Tyr Gly Trp Ile Thr
            180                 185                 190

atc aac tat ctg ctg ggc aaa ttc agt cag aaa aca agg tgg ttc agc   624
Ile Asn Tyr Leu Leu Gly Lys Phe Ser Gln Lys Thr Arg Trp Phe Ser
        195                 200                 205

ata gtc cca tat gaa acc aat aat cag gaa acc ttt gga gct ttg gac   672
Ile Val Pro Tyr Glu Thr Asn Asn Gln Glu Thr Phe Gly Ala Leu Asp
    210                 215                 220

ctt ggg gga gcc tct aca caa gtc act ttt gta ccc caa aac cag act   720
Leu Gly Gly Ala Ser Thr Gln Val Thr Phe Val Pro Gln Asn Gln Thr
225                 230                 235                 240

atc gag tcc cca gat aat gct ctg caa ttt cgc ctc tat ggc aag gac   768
Ile Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg Leu Tyr Gly Lys Asp
                245                 250                 255

tac aat gtc tac aca cat agc ttc ttg tgc tat ggg aag gat cag gca   816
Tyr Asn Val Tyr Thr His Ser Phe Leu Cys Tyr Gly Lys Asp Gln Ala
            260                 265                 270

ctc tgg cag aaa ctg gcc aag gac att cag gtt gca agt aat gaa att   864
Leu Trp Gln Lys Leu Ala Lys Asp Ile Gln Val Ala Ser Asn Glu Ile
        275                 280                 285

ctc agg gac cca tgc ttt cat cct gga tat aag aag gta gtg aac gta   912
Leu Arg Asp Pro Cys Phe His Pro Gly Tyr Lys Lys Val Val Asn Val
    290                 295                 300

agt gac ctt tac aag acc ccc tgc acc aag aga ttt gag atg act ctt   960
Ser Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg Phe Glu Met Thr Leu
305                 310                 315                 320

cca ttc cag cag ttt gaa atc cag ggt att gga aac tat caa caa tgc  1008
Pro Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly Asn Tyr Gln Gln Cys
                325                 330                 335

cat caa agc atc ctg gag ctc ttc aac acc agt tac tgc cct tac tcc  1056
His Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser Tyr Cys Pro Tyr Ser
            340                 345                 350

cag tgt gcc ttc aat ggg att ttc ttg cca cca ctc cag ggg gat ttt  1104
Gln Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro Leu Gln Gly Asp Phe
        355                 360                 365

ggg gca ttt tca gct ttt tac ttt gtg atg aag ttt tta aac ttg aca  1152
Gly Ala Phe Ser Ala Phe Tyr Phe Val Met Lys Phe Leu Asn Leu Thr
    370                 375                 380

tca gag aaa gtc tct cag gaa aag gtg act gag atg atg aaa aag ttc  1200
```

```
Ser Glu Lys Val Ser Gln Glu Lys Val Thr Glu Met Met Lys Lys Phe
385                 390                 395                 400

tgt gct cag cct tgg gag gag ata aaa aca tct tac gct gga gta aag   1248
Cys Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser Tyr Ala Gly Val Lys
                405                 410                 415

gag aag tac ctg agt gaa tac tgc ttt tct ggt acc tac att ctc tcc   1296
Glu Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly Thr Tyr Ile Leu Ser
            420                 425                 430

ctc ctt ctg caa ggc tat cat ttc aca gct gat tcc tgg gag cac atc   1344
Leu Leu Leu Gln Gly Tyr His Phe Thr Ala Asp Ser Trp Glu His Ile
        435                 440                 445

cat ttc att ggc aag atc cag ggc agc gac gcc ggc tgg act ttg ggc   1392
His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala Gly Trp Thr Leu Gly
    450                 455                 460

tac atg ctg aac ctg acc aac atg atc cca gct gag caa cca ttg tcc   1440
Tyr Met Leu Asn Leu Thr Asn Met Ile Pro Ala Glu Gln Pro Leu Ser
465                 470                 475                 480

aca cct ctc tcc cac tcc acc taa                                  1464
Thr Pro Leu Ser His Ser Thr
                485
```

<210> 26
<211> 487
<212> PRT
<213> Artificial Sequence

<400> 26

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Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1               5                  10                  15

Ser Leu Ala Leu Val Thr Asn Ser Ala Pro Thr Ser Ser Ser Thr Lys
            20                  25                  30

Lys Thr Gln Leu Thr Ser Ser Gly Asp Tyr Lys Asp Asp Asp Asp Lys
        35                  40                  45

Thr Gln Asn Lys Ala Leu Pro Glu Asn Val Lys Tyr Gly Ile Val Leu
    50                  55                  60

Asp Ala Gly Ser Ser His Thr Ser Leu Tyr Ile Tyr Lys Trp Pro Ala
65                  70                  75                  80

Glu Lys Glu Asn Asp Thr Gly Val Val His Gln Val Glu Glu Cys Arg
                85                  90                  95

Val Lys Gly Pro Gly Ile Ser Lys Phe Val Gln Lys Val Asn Glu Ile
            100                 105                 110

Gly Ile Tyr Leu Thr Asp Cys Met Glu Arg Ala Arg Glu Val Ile Pro
        115                 120                 125
```

```
Arg Ser Gln His Gln Glu Thr Pro Val Tyr Leu Gly Ala Thr Ala Gly
    130                 135                 140

Met Arg Leu Leu Arg Met Glu Ser Glu Glu Leu Ala Asp Arg Val Leu
145                 150                 155                 160

Asp Val Val Glu Arg Ser Leu Ser Asn Tyr Pro Phe Asp Phe Gln Gly
                165                 170                 175

Ala Arg Ile Ile Thr Gly Gln Glu Glu Gly Ala Tyr Gly Trp Ile Thr
            180                 185                 190

Ile Asn Tyr Leu Leu Gly Lys Phe Ser Gln Lys Thr Arg Trp Phe Ser
        195                 200                 205

Ile Val Pro Tyr Glu Thr Asn Asn Gln Glu Thr Phe Gly Ala Leu Asp
    210                 215                 220

Leu Gly Gly Ala Ser Thr Gln Val Thr Phe Val Pro Gln Asn Gln Thr
225                 230                 235                 240

Ile Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg Leu Tyr Gly Lys Asp
                245                 250                 255

Tyr Asn Val Tyr Thr His Ser Phe Leu Cys Tyr Gly Lys Asp Gln Ala
            260                 265                 270

Leu Trp Gln Lys Leu Ala Lys Asp Ile Gln Val Ala Ser Asn Glu Ile
        275                 280                 285

Leu Arg Asp Pro Cys Phe His Pro Gly Tyr Lys Lys Val Val Asn Val
    290                 295                 300

Ser Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg Phe Glu Met Thr Leu
305                 310                 315                 320

Pro Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly Asn Tyr Gln Gln Cys
                325                 330                 335

His Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser Tyr Cys Pro Tyr Ser
            340                 345                 350

Gln Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro Leu Gln Gly Asp Phe
        355                 360                 365

Gly Ala Phe Ser Ala Phe Tyr Phe Val Met Lys Phe Leu Asn Leu Thr
    370                 375                 380

Ser Glu Lys Val Ser Gln Glu Lys Val Thr Glu Met Met Lys Lys Phe
385                 390                 395                 400

Cys Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser Tyr Ala Gly Val Lys
                405                 410                 415

Glu Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly Thr Tyr Ile Leu Ser
            420                 425                 430
```

```
Leu Leu Leu Gln Gly Tyr His Phe Thr Ala Asp Ser Trp Glu His Ile
        435                 440                 445

His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala Gly Trp Thr Leu Gly
    450                 455                 460

Tyr Met Leu Asn Leu Thr Asn Met Ile Pro Ala Glu Gln Pro Leu Ser
465                 470                 475                 480

Thr Pro Leu Ser His Ser Thr
                485
```

<210> 27
<211> 464
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 27

```
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1               5                  10                  15

Ser Leu Ala Leu Val Thr Asn Ser Ala Thr Gln Asn Lys Ala Leu Pro
             20                  25                  30

Glu Asn Val Lys Tyr Gly Ile Val Leu Asp Ala Gly Ser Ser His Thr
         35                  40                  45

Ser Leu Tyr Ile Tyr Lys Trp Pro Ala Glu Lys Glu Asn Asp Thr Gly
     50                  55                  60

Val Val His Gln Val Glu Glu Cys Arg Val Lys Gly Pro Gly Ile Ser
 65                  70                  75                  80

Lys Phe Val Gln Lys Val Asn Glu Ile Gly Ile Tyr Leu Thr Asp Cys
                 85                  90                  95

Met Glu Arg Ala Arg Glu Val Ile Pro Arg Ser Gln His Gln Glu Thr
            100                 105                 110

Pro Val Tyr Leu Gly Ala Thr Ala Gly Met Arg Leu Leu Arg Met Glu
        115                 120                 125

Ser Glu Glu Leu Ala Asp Arg Val Leu Asp Val Val Glu Arg Ser Leu
    130                 135                 140

Ser Asn Tyr Pro Phe Asp Phe Gln Gly Ala Arg Ile Ile Thr Gly Gln
145                 150                 155                 160

Glu Glu Gly Ala Tyr Gly Trp Ile Thr Ile Asn Tyr Leu Leu Gly Lys
                165                 170                 175

Phe Ser Gln Lys Thr Arg Trp Phe Ser Ile Val Pro Tyr Glu Thr Asn
            180                 185                 190
```

```
Asn Gln Glu Thr Phe Gly Ala Leu Asp Leu Gly Gly Ala Ser Thr Gln
        195                 200                 205

Val Thr Phe Val Pro Gln Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala
    210                 215                 220

Leu Gln Phe Arg Leu Tyr Gly Lys Asp Tyr Asn Val Tyr Thr His Ser
225                 230                 235                 240

Phe Leu Cys Tyr Gly Lys Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys
                245                 250                 255

Asp Ile Gln Val Ala Ser Asn Glu Ile Leu Arg Asp Pro Cys Phe His
            260                 265                 270

Pro Gly Tyr Lys Lys Val Val Asn Val Ser Asp Leu Tyr Lys Thr Pro
        275                 280                 285

Cys Thr Lys Arg Phe Glu Met Thr Leu Pro Phe Gln Gln Phe Glu Ile
    290                 295                 300

Gln Gly Ile Gly Asn Tyr Gln Gln Cys His Gln Ser Ile Leu Glu Leu
305                 310                 315                 320

Phe Asn Thr Ser Tyr Cys Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile
                325                 330                 335

Phe Leu Pro Pro Leu Gln Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr
            340                 345                 350

Phe Val Met Lys Phe Leu Asn Leu Thr Ser Glu Lys Val Ser Gln Glu
        355                 360                 365

Lys Val Thr Glu Met Met Lys Lys Phe Cys Ala Gln Pro Trp Glu Glu
    370                 375                 380

Ile Lys Thr Ser Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr
385                 390                 395                 400

Cys Phe Ser Gly Thr Tyr Ile Leu Ser Leu Leu Leu Gln Gly Tyr His
                405                 410                 415

Phe Thr Ala Asp Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln
            420                 425                 430

Gly Ser Asp Ala Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn
        435                 440                 445

Met Ile Pro Ala Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr
    450                 455                 460
```

<210> 28
<211> 474

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 28

```
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1               5                  10                  15

Ser Leu Ala Leu Val Thr Asn Ser Ala Ser Thr Lys Lys Thr Gln Leu
            20                  25                  30

Thr Ser Ser Thr Gln Asn Lys Ala Leu Pro Glu Asn Val Lys Tyr Gly
        35                  40                  45

Ile Val Leu Asp Ala Gly Ser Ser His Thr Ser Leu Tyr Ile Tyr Lys
    50                  55                  60

Trp Pro Ala Glu Lys Glu Asn Asp Thr Gly Val Val His Gln Val Glu
65                  70                  75                  80

Glu Cys Arg Val Lys Gly Pro Gly Ile Ser Lys Phe Val Gln Lys Val
                85                  90                  95

Asn Glu Ile Gly Ile Tyr Leu Thr Asp Cys Met Glu Arg Ala Arg Glu
            100                 105                 110

Val Ile Pro Arg Ser Gln His Gln Glu Thr Pro Val Tyr Leu Gly Ala
        115                 120                 125

Thr Ala Gly Met Arg Leu Leu Arg Met Glu Ser Glu Glu Leu Ala Asp
    130                 135                 140

Arg Val Leu Asp Val Val Glu Arg Ser Leu Ser Asn Tyr Pro Phe Asp
145                 150                 155                 160

Phe Gln Gly Ala Arg Ile Ile Thr Gly Gln Glu Glu Gly Ala Tyr Gly
                165                 170                 175

Trp Ile Thr Ile Asn Tyr Leu Leu Gly Lys Phe Ser Gln Lys Thr Arg
            180                 185                 190

Trp Phe Ser Ile Val Pro Tyr Glu Thr Asn Asn Gln Glu Thr Phe Gly
        195                 200                 205

Ala Leu Asp Leu Gly Gly Ala Ser Thr Gln Val Thr Phe Val Pro Gln
    210                 215                 220

Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg Leu Tyr
225                 230                 235                 240

Gly Lys Asp Tyr Asn Val Tyr Thr His Ser Phe Leu Cys Tyr Gly Lys
                245                 250                 255

Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp Ile Gln Val Ala Ser
            260                 265                 270
```

```
Asn Glu Ile Leu Arg Asp Pro Cys Phe His Pro Gly Tyr Lys Lys Val
        275                 280                 285

Val Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg Phe Glu
    290                 295                 300

Met Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly Asn Tyr
305                 310                 315                 320

Gln Gln Cys His Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser Tyr Cys
                325                 330                 335

Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro Leu Gln
            340                 345                 350

Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe Val Met Lys Phe Leu
        355                 360                 365

Asn Leu Thr Ser Glu Lys Val Ser Gln Glu Lys Val Thr Glu Met Met
    370                 375                 380

Lys Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser Tyr Ala
385                 390                 395                 400

Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly Thr Tyr
                405                 410                 415

Ile Leu Ser Leu Leu Leu Gln Gly Tyr His Phe Thr Ala Asp Ser Trp
            420                 425                 430

Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala Gly Trp
        435                 440                 445

Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile Pro Ala Glu Gln
    450                 455                 460

Pro Leu Ser Thr Pro Leu Ser His Ser Thr
465                 470


<210> 29
<211> 473
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:  Fusion
      construct of human CD39

<400> 29
Met Ala Leu Trp Ile Asp Arg Met Gln Leu Leu Ser Cys Ile Ala Leu
  1                 5                  10                  15

Ser Leu Ala Leu Val Thr Asn Ser Ser Thr Lys Lys Thr Gln Leu Thr
             20                  25                  30
```

Ser Ser Thr Gln Asn Lys Ala Leu Pro Glu Asn Val Lys Tyr Gly Ile
35 40 45

Val Leu Asp Ala Gly Ser Ser His Thr Ser Leu Tyr Ile Tyr Lys Trp
50 55 60

Pro Ala Glu Lys Glu Asn Asp Thr Gly Val Val His Gln Val Glu Glu
65 70 75 80

Cys Arg Val Lys Gly Pro Gly Ile Ser Lys Phe Val Gln Lys Val Asn
85 90 95

Glu Ile Gly Ile Tyr Leu Thr Asp Cys Met Glu Arg Ala Arg Glu Val
100 105 110

Ile Pro Arg Ser Gln His Gln Glu Thr Pro Val Tyr Leu Gly Ala Thr
115 120 125

Ala Gly Met Arg Leu Leu Arg Met Glu Ser Glu Glu Leu Ala Asp Arg
130 135 140

Val Leu Asp Val Val Glu Arg Ser Leu Ser Asn Tyr Pro Phe Asp Phe
145 150 155 160

Gln Gly Ala Arg Ile Ile Thr Gly Gln Glu Glu Gly Ala Tyr Gly Trp
165 170 175

Ile Thr Ile Asn Tyr Leu Leu Gly Lys Phe Ser Gln Lys Thr Arg Trp
180 185 190

Phe Ser Ile Val Pro Tyr Glu Thr Asn Asn Gln Glu Thr Phe Gly Ala
195 200 205

Leu Asp Leu Gly Gly Ala Ser Thr Gln Val Thr Phe Val Pro Gln Asn
210 215 220

Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg Leu Tyr Gly
225 230 235 240

Lys Asp Tyr Asn Val Tyr Thr His Ser Phe Leu Cys Tyr Gly Lys Asp
245 250 255

Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp Ile Gln Val Ala Ser Asn
260 265 270

Glu Ile Leu Arg Asp Pro Cys Phe His Pro Gly Tyr Lys Lys Val Val
275 280 285

Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg Phe Glu Met
290 295 300

Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly Asn Tyr Gln
305 310 315 320

Gln Cys His Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser Tyr Cys Pro
325 330 335

Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro Leu Gln Gly
340 345 350

Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe Val Met Lys Phe Leu Asn
355 360 365

Leu Thr Ser Glu Lys Val Ser Gln Glu Lys Val Thr Glu Met Met Lys
370 375 380

Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser Tyr Ala Gly
385 390 395 400

Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly Thr Tyr Ile
405 410 415

Leu Ser Leu Leu Leu Gln Gly Tyr His Phe Thr Ala Asp Ser Trp Glu
420 425 430

His Ile His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala Gly Trp Thr
435 440 445

Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile Pro Ala Glu Gln Pro
450 455 460

Leu Ser Thr Pro Leu Ser His Ser Thr
465 470

<210> 30
<211> 463
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Fusion construct of human CD39

<400> 30
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
1 5 10 15

Gly Ser Thr Gly Ala Pro Thr Ser Thr Gln Asn Lys Ala Leu Pro Glu
20 25 30

Asn Val Lys Tyr Gly Ile Val Leu Asp Ala Gly Ser Ser His Thr Ser
35 40 45

Leu Tyr Ile Tyr Lys Trp Pro Ala Glu Lys Glu Asn Asp Thr Gly Val
50 55 60

Val His Gln Val Glu Glu Cys Arg Val Lys Gly Pro Gly Ile Ser Lys
65 70 75 80

Phe Val Gln Lys Val Asn Glu Ile Gly Ile Tyr Leu Thr Asp Cys Met
85 90 95

Glu Arg Ala Arg Glu Val Ile Pro Arg Ser Gln His Gln Glu Thr Pro
100 105 110

Val Tyr Leu Gly Ala Thr Ala Gly Met Arg Leu Leu Arg Met Glu Ser
115 120 125

Glu Glu Leu Ala Asp Arg Val Leu Asp Val Val Glu Arg Ser Leu Ser
130 135 140

Asn Tyr Pro Phe Asp Phe Gln Gly Ala Arg Ile Ile Thr Gly Gln Glu
145 150 155 160

Glu Gly Ala Tyr Gly Trp Ile Thr Ile Asn Tyr Leu Leu Gly Lys Phe
165 170 175

Ser Gln Lys Thr Arg Trp Phe Ser Ile Val Pro Tyr Glu Thr Asn Asn
180 185 190

Gln Glu Thr Phe Gly Ala Leu Asp Leu Gly Gly Ala Ser Thr Gln Val
195 200 205

Thr Phe Val Pro Gln Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu
210 215 220

Gln Phe Arg Leu Tyr Gly Lys Asp Tyr Asn Val Tyr Thr His Ser Phe
225 230 235 240

Leu Cys Tyr Gly Lys Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp
245 250 255

Ile Gln Val Ala Ser Asn Glu Ile Leu Arg Asp Pro Cys Phe His Pro
260 265 270

Gly Tyr Lys Lys Val Val Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys
275 280 285

Thr Lys Arg Phe Glu Met Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln
290 295 300

Gly Ile Gly Asn Tyr Gln Gln Cys His Gln Ser Ile Leu Glu Leu Phe
305 310 315 320

Asn Thr Ser Tyr Cys Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe
325 330 335

Leu Pro Pro Leu Gln Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe
340 345 350

Val Met Lys Phe Leu Asn Leu Thr Ser Glu Lys Val Ser Gln Glu Lys
355 360 365

Val Thr Glu Met Met Lys Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile
370 375 380

Lys Thr Ser Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys
385 390 395 400

Phe Ser Gly Thr Tyr Ile Leu Ser Leu Leu Leu Gln Gly Tyr His Phe
405 410 415

```
Thr Ala Asp Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly
            420                 425                 430

Ser Asp Ala Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met
        435                 440                 445

Ile Pro Ala Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr
    450                 455                 460
```

<210> 31
<211> 58
<212> PRT
<213> Homo sapiens

<400> 31

```
Met Ala Thr Ser Trp Gly Thr Val Phe Phe Met Leu Val Val Ser Cys
  1               5                  10                  15

Val Cys Ser Ala Val Ser His Arg Asn Gln Gln Thr Trp Phe Glu Gly
             20                  25                  30

Ile Phe Leu Ser Ser Met Cys Pro Ile Asn Val Ser Ala Ser Thr Leu
         35                  40                  45

Tyr Gly Ile Met Phe Asp Ala Gly Ser Thr
     50                  55
```

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Confirmation

Confirmation

December 13, 2000

IN THE INTERNATIONAL PRELIMINARY EXAMINING
AUTHORITY OF THE PATENT COOPERATION TREATY

In Re: International Application of Immunex Corporation et al.
Application No. PCT/US99/23641
Filed October 13, 1999

Authorized Officer:
F. Perez

METHODS OF INHIBITING
PLATELET ACTIVATION AND RECRUITMENT

Attorney Docket No. 23,495 PCT

CERTIFICATE OF FACSIMILE TRANSMISSION AND MAILING

I hereby certify that this correspondence is being transmitted by facsimile, on **December 13, 2000**, to: European Patent Office, D-80298 Munich, Germany at facsimile no. 011 49 89 2399-4465. A confirmation copy of this correspondence follows by airmail.

Lynn M. White
Lynn M. White

European Patent Office
D-80298 Munich
Germany

Attention: IPEA/EP

REPLY UNDER PCT ARTICLE 34 §(2)(d)
TO WRITTEN OPINION DATED AUGUST 18, 2000

Sir:

This is in response to the Written Opinion dated August 18, 2000. Applicant amends as follows.

In the Specification

Page 2, line 6, insert --; WO 96/30532-- between "1998" and ")".

In the Claims:

1. (Amended) A method for inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide having a structure X-Y wherein X is selected from the group consisting of an Ala residue and heterologous peptides capable of adopting a stable secondary structure and Y is selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in [Figure 1] (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; and

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity[; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides have apyrase activity].

2. (Amended) The method of claim 1 wherein Y [the polypeptide] is selected from the group consisting of:

(a) polypeptides having a sequence consisting of amino acids 38-476 or 39-476 of SEQ ID NO:2;

(b) variant polypeptides that are at least 70% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(c) variant polypeptides that are at least 80% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(d) variant polypeptides that are at least 90% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(e) variant polypeptides that are at least 95% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(f) variant polypeptides that are at least 98% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity; and

(g) variant polypeptides that are at least 99% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity.

Cancel Claim 3.

Renumber Claims 4 to 6 as Claims 3 to 5, respectively.

3 [4]. (Amended) The method of claim 1 [3] wherein X is a peptide fragment from the amino terminal portion of mature IL-2, CD39-L2, CD39-L3, or CD39-L4.

4 [5]. (Amended) The method of claim 1 comprising administering [A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of] a polypeptide having the structure A-B-Y [A-B-C] wherein A is 0-20 amino acids from the amino terminal portion of mature IL-2[,] and B is a linker of 0-15 amino acids[, and C is the soluble CD39 polypeptide of claim 1].

5 [6]. (Amended) A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide selected from the group consisting of:

(a) SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, or amino acids 21-463 of SEQ ID NO:30; and

(b) [fragments of the polypeptides of (a) wherein said fragments have apyrase activity;

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and

(d)] fusion polypeptides comprising the polypeptides of (a)[, (b), or (c)], wherein said fusion polypeptides have apyrase activity.

Cancel claim 7.

Renumber claim 8 as claim 6.

6 [8]. (Amended) The method of claim 5 [6] wherein the soluble CD39 polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, and amino acids 21-463 of SEQ ID NO:30.

Insert as new claim 7 the following:

--7. The method of claim 6 wherein the soluble CD39 polypeptide has the sequence of amino acids 21-463 of SEQ ID NO: 30.--

Renumber claims 9 through 20 as claims 8 through 19, respectively.

8 [9]. (Amended) A method according to one of claims 1-7 wherein the [of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a] soluble CD39 polypeptide [that] has

been produced by culturing a recombinant cell that encodes the [a] soluble CD39 polypeptide [according to claim 1] under conditions permitting expression of the CD39 polypeptide, and recovering the expressed CD39 polypeptide.

9 [10]. (Amended) The method of claim 8 [9] wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:5; and

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:5[;

(c) DNA sequences that hybridize to SEQ ID NO:5 under moderately stringent conditions;

(d) DNA sequences that are at least 70% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(e) DNA sequences that are at least 80% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(f) DNA sequences that are at least 90% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(g) DNA sequences that are at least 95% identical in sequence to SEQ ID NO:5 or to a fragment thereof;

(h) DNA sequences that are at least 98% identical in sequence to SEQ ID NO:5 or to a fragment thereof; and

(i) DNA sequences that are at least 99% identical in sequence to SEQ ID NO:5 or to a fragment thereof].

10 [11]. The method of claim 8 [9] wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:7; and

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:7[;

(c) DNA sequences which hybridize to SEQ ID NO:7 under moderately stringent conditions;

(d) DNA sequences that are at least 70% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(e) DNA sequences that are at least 80% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(f) DNA sequences that are at least 90% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(g) DNA sequences that are at least 95% identical in sequence to SEQ ID NO:7 or to a fragment thereof;

(h) DNA sequences that are at least 98% identical in sequence to SEQ ID NO:7 or to a fragment thereof; and

(i) DNA sequences that are at least 99% identical in sequence to SEQ ID NO:7 or to a fragment thereof].

11 [12]. (Amended) The method of one of claims 1-10 [1-11] wherein the soluble CD39 polypeptide is administered in a composition comprising a pharmaceutically acceptable carrier.

12 [13]. The method of one of claims 1-11 wherein the soluble CD39 polypeptide is administered in combination with at least one other antithrombotic or antiplatelet composition.

13 [14]. (Amended) The method of claim 12 [13] wherein the soluble CD39 polypeptide is administered in combination with aspirin.

14 [15]. (Amended) The method of one of claims 1-13 [1-11] wherein the soluble CD39 polypeptide is administered parenterally.

15 [16]. (Amended) The method of claim 14 [15] wherein the soluble CD39 polypeptide is administered intravenously.

16 [17]. (Amended) The method of one of claims 1-15 [1-11] wherein the mammal is suffering from unstable angina, myocardial infarction, stroke, coronary artery disease or injury, myocardial infarction, atherosclerosis, peripheral vascular occlusion, preeclampsia, embolism, a platelet-associated ischemic disorder including lung ischemia, coronary ischemia, and cerebral ischemia, a thrombotic disorder including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombosis and coagulopathy associated with exposure to a foreign or injured tissue surface, deep venous thrombosis (DVT), pulmonary embolism (PE), transient ischemic attack (TIAs), or another related condition where vascular occlusion is the common underlying feature.

17 [18]. (Amended) The method of one of claims 1-15 [1-11] wherein the soluble CD39 is administered to prevent thrombus formation or reformation, occlusion, reocclusion, stenosis, or restenosis of blood vessels, or stroke.

18 [19]. (Amended) The method of one of claims 1-15 [1-11] wherein the soluble CD39 is administered in conjunction with angioplasty, carotid endarterectomy, anastomosis of vascular graft, atherectomy, stent placement, placement of a chronic cardiovascular device such as an in-dwelling catheter or prosthetic valve or vessel, or bypass surgery.

19 [20]. (Amended) A method for degrading nucleoside tri- and/or di- phosphates in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide having a structure X-Y wherein X is selected from the group consisting of an Ala residue and heterologous peptides capable of adopting a stable secondary structure and Y is selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in [Figure 1] (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; and

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity[; and

(d) fusion polypeptides comprising the polypeptides of (a), (b), or (c), wherein said fusion polypeptides have apyrase activity].

Insert new page 58 which now contains the Abstract.

Delete page 59 in its entirety.

REMARKS

Reconsideration of the allowability of the claims of the present application is requested respectfully.

Status of the Claims

The Written Opinion of August 18, 2000 addresses all of the claims of the present application, that is, claims 1 to 20. Claims 1, 2, 4 to 6, 8 to 12, and 14 to 20 have been amended, claims 3 and 7 have been canceled, and a new claim 7 has been added.

Summary of the Authorized Officer's Statements

A. Statements Regarding Novelty

Applicants believe that the presently amended claims are novel. Support for the amendment to Claims 1 and 19 (now Claim 20) may be found throughout the specification and claims as filed and, in particular, at page 11, lines 21 to 26.

B. Statement Regarding Inventiveness

With regard to the Authorized Officer's statement that claims 1 to 20 lack an inventive step under Articles 33.1 and 33.3 PCT Applicants submit that methods for using a novel, inventive and industrial applicable composition is a patentable invention. The presently amended claims all define methods for using novel and non-obvious compositions. Accordingly, Applicants submit that methods for using these novel and non-obvious compositions are not obvious. Furthermore, Applicants submit that the use of the presently claimed fusion polypeptides is not foreseen by the disclosure in document D5. The D5 reference relates to replacement of a natural interleukin-2 (IL-2) mRNA 5' non-coding region with a leader element derived from an efficiently translated rat preproinsulin II mRNA. The D5 reference describes modifications to the 5'-non-coding region and to the N-terminal portion of a signal peptide (which is removed upon secretion from the cell), whereas the present invention relates to the use of soluble CD39 fusion polypeptides. There is no disclosure in the D5 reference of CD39 nor is there any mention of methods of treatment utilizing soluble CD39 fusion polypeptides. The D5 reference simply demonstrates that a certain leader sequence may be used to enhance expression of IL-2. Contrary to the Authorized Officer's statements, the D1 reference does not strongly suggest use of the currently claimed fusion CD39 polypeptides since the presently claimed fusion polypeptides are not disclosed in D1.

C. Statement Regarding Item VIII

Applicants have amended the description by adding a reference to document D2 --; WO 96/30532-- between "1998" and ")" at page 2, line 6. Applicants do not believe that document D5, which describes the use of heterologous mRNA leader and signal peptide regions in the expression of IL-2, is relevant background art with respect to the present invention.

D. Statement Regarding Item VIII

The reference to Figure 1 has been deleted from claim 1, which now refers directly to SEQ ID NO: 2.

The number of independent claims has been reduced (Claims 3 and 7 have been canceled and Claims 5 and 9, renumbered as 4 and 8, have been drafted in dependent form) in response to the observations regarding conciseness.

Applicant has included with this Reply replacement pages 2, and 54 to 58 which incorporate the amendments included in the Reply. Applicant has canceled page 59 which became unnecessary in view of the proposed amendments and replacement pages provided. All of the claims are presented on pages 54 to 57 and the Abstract appears on page 58.

Respectfully submitted,

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Immunoprecipitation of HUVEC detergent lysates with anti-CD39 mAb resulted in complete capture of cell-associated ADPase activity, suggesting that CD39 is the only ecto-ADPase on endothelial cells (Marcus et al., *J. Clin. Invest.* 99:1351, 1997). In the same study, COS cell transfectants expressing recombinant CD39 at the cell surface totally inhibited ADP-induced platelet aggregation. Thus, CD39 plays a prominent role in thromboregulation (*see also,* Gayle et al., *J. Clin. Invest.*, 101:1851, 1998; WO96/30532).

Excessive platelet activation (i.e., stimulation by an agonist) and recruitment, leading to platelet aggregation and vessel occlusion at sites of vascular injury in the coronary, carotid, and peripheral arteries, presents a major therapeutic challenge in cardiovascular medicine. Excessive platelet activation and recruitment is a contributing factor in clinical disorders including stroke, unstable angina, myocardial infarction, and restenosis following percutaneous coronary intervention including angioplasty, atherectomy, stent placement, and bypass surgery.

Glycoprotein IIb/IIIa antagonists, such as the monoclonal antibody marketed as ReoPro® (Centocor Inc.), are presently under development for the inhibition of platelet aggregation in patients undergoing percutaneous coronary intervention, and in patients with acute coronary syndromes such as unstable angina and myocardial infarction. The activation of glycoprotein IIb/IIIa receptors, however, is a late event in the cascade that leads to platelet aggregation.

There is a great need to identify additional therapeutic strategies and compositions for the pharmacological neutralization of platelet reactivity (activation, recruitment, aggregation). In particular, there is a need to identify compounds and compositions which target early portions of coagulation pathways such as the ADP-dependent activation and recruitment of platelets. There is, in fact, an urgent need to identify new strategies and compositions for the treatment of stroke, which is the third leading cause of death in the United States. In the case of stroke, an advantageous therapeutic agent will reduce intravascular thrombus burden and accompanying neurological defects without increasing intracerebral hemorrhage.

SUMMARY OF THE INVENTION

Soluble forms of CD39 having apyrase activity constitute a novel approach to the prevention and/or treatment of disease. The present invention provides soluble CD39 polypeptides and nucleic acids, compositions comprising a pharmaceutically acceptable carrier and a soluble CD39 polypeptide, and methods of making and using soluble CD39 polypeptides having apyrase activity. The effectiveness of soluble CD39 polypeptides have been demonstrated in vitro, ex vivo, and in vivo.

The invention is directed to soluble CD39 polypeptides selected from the group consisting of: (a) polypeptides having an amino acid sequence as set forth in Figure 1 (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478; (b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; (c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and (d) fusion polypeptides comprising the polypeptides of (a), (b),

CLAIMS

We claim:

1. A method for inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide having a structure X-Y wherein X is selected from the group consisting of an Ala residue and heterologous peptides capable of adopting a stable secondary structure and Y is selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; and

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity.

2. The method of claim 1 wherein Y is selected from the group consisting of:

(a) polypeptides having a sequence consisting of amino acids 38-476 or 39-476 of SEQ ID NO:2;

(b) variant polypeptides that are at least 70% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(c) variant polypeptides that are at least 80% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(d) variant polypeptides that are at least 90% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(e) variant polypeptides that are at least 95% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(f) variant polypeptides that are at least 98% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity; and

(g) variant polypeptides that are at least 99% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity.

3. The method of claim 1 wherein X is a peptide fragment from the amino terminal portion of mature IL-2, CD39-L2, CD39-L3, or CD39-L4.

4. The method of claim 1 comprising administering a polypeptide having the structure A-B-Y wherein A is 0-20 amino acids from the amino terminal portion of mature IL-2 and B is a linker of 0-15 amino acids.

5. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide selected from the group consisting of:

(a) SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, or amino acids 21-463 of SEQ ID NO:30; and

(b) fusion polypeptides comprising the polypeptides of (a), wherein said fusion polypeptides have apyrase activity.

6. The method of claim 5 wherein the soluble CD39 polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, and amino acids 21-463 of SEQ ID NO:30.

7. The method of claim 6 wherein the soluble CD39 polypeptide has the sequence of amino acids 21-463 of SEQ ID NO: 30.

8. A method according to one of claims 1-7 wherein the soluble CD39 polypeptide has been produced by culturing a recombinant cell that encodes the soluble CD39 polypeptide under conditions permitting expression of the CD39 polypeptide, and recovering the expressed CD39 polypeptide.

9. The method of claim 8 wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:5; and

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:5.

10. The method of claim 8 wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:7; and

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:7.

11. The method of one of claims 1-10 wherein the soluble CD39 polypeptide is administered in a composition comprising a pharmaceutically acceptable carrier.

12. The method of one of claims 1-11 wherein the soluble CD39 polypeptide is administered in combination with at least one other antithrombotic or antiplatelet composition.

13. The method of claim 12 wherein the soluble CD39 polypeptide is administered in combination with aspirin.

14. The method of one of claims 1-13 wherein the soluble CD39 polypeptide is administered parenterally.

15. The method of claim 14 wherein the soluble CD39 polypeptide is administered intravenously.

16. The method of one of claims 1-15 wherein the mammal is suffering from unstable angina, myocardial infarction, stroke, coronary artery disease or injury, myocardial infarction, atherosclerosis, peripheral vascular occlusion, preeclampsia, embolism, a platelet-associated ischemic disorder including lung ischemia, coronary ischemia, and cerebral ischemia, a thrombotic disorder including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombosis and coagulopathy associated with exposure to a foreign or injured tissue surface, deep venous thrombosis (DVT), pulmonary embolism (PE), transient ischemic attack (TIAs), or another related condition where vascular occlusion is the common underlying feature.

17. The method of one of claims 1-15 wherein the soluble CD39 is administered to prevent thrombus formation or reformation, occlusion, reocclusion, stenosis, or restenosis of blood vessels, or stroke.

18. The method of one of claims 1-15 wherein the soluble CD39 is administered in conjunction with angioplasty, carotid endarterectomy, anastomosis of vascular graft, atherectomy, stent placement, placement of a chronic cardiovascular device such as an in-dwelling catheter or prosthetic valve or vessel, or bypass surgery.

19. A method for degrading nucleoside tri- and/or di- phosphates in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide having a structure X-Y wherein X is selected from the group consisting of an Ala residue and heterologous peptides capable of adopting a stable secondary structure and Y is selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; and

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity.

METHODS OF INHIBITING PLATELET ACTIVATION AND RECRUITMENT

ABSTRACT OF THE INVENTION

The present invention provides soluble CD39 polypeptides and compositions, and methods for inhibiting platelet activation and recruitment in a mammal comprising administering a soluble CD39 polypeptide.

PATENT COOPERATION TREATY

ENTERED COMPUTER
11-18-00

From the:
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
KELLY,P
SYNNESTVEDT & LECHNER
2600 ARAMARK Tower
1101 Market Street
Philadelphia, PA 19107-2950
ETATS-UNIS D'AMERIQUE

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of mailing *(day/month/year)*	18.08.2000
Applicant's or agent's file reference: P23,495 PCT	**REPLY DUE** **within 3 month(s)** from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23641	13/10/1999	16/10/1998

International Patent Classification (IPC) or both national classification and IPC
A61K38/17

Applicant
IMMUNEX CORPORATION et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain document cited
VII	☒	Certain defects in the international application
VIII	☒	Certain observations on the international application

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SYNNESTVEDT & LECHNER
WRITTEN: PJK

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 16/02/2001.

Name and mailing address of the international preliminary examining authority:
European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized officer / Examiner
Perez, F

Formalities officer (incl. extension of time limits)
Hundt, D
Telephone No. +49 89 2399 8042

I. Basis of the opinion

1. This opinion has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*):

Description, pages:

1-53 as originally filed

Claims, No.:

1-20 as originally filed

Drawings, sheets:

1/24-24/24 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-20,

because:

☒ the said international application, or the said claims Nos. 1-20 (inventive step) relate to the following subject matter which does not require an international preliminary examination (*specify*):

see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-2, 6-7, 9-12, 15-20 (NO)
Inventive step (IS)	Claims	1-20 (NO)
Industrial applicability (IA)	Claims	see separate sheet

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1) **Claims 1-20** relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34.4(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2) Reference is made to the following documents:

D1: GAYLE RICHARD B III ET AL. JOURNAL OF CLINICAL INVESTIGATION MAY 1, 1998, vol. 101, no. 9, 1 May 1998, pages 1851-1859.
D2: WO 96 30532 A (SANDOZ LTD ;NEW ENGLAND DEACONESS HOSPITAL (US); BACH FRITZ H (US)) 3 October 1996.
D3: CHADWICK B P ET AL. GENOMICS,US,ACADEMIC PRESS, SAN DIEGO, vol. 50, no. 3, 15 June 1998 (1998-06-15), pages 357-367.
D4: MARCUS AARON J ET AL. JOURNAL OF CLINICAL INVESTIGATION 1997, vol. 99, no. 6, 1997, pages 1351-1360.
D5: CULLEN B. R. DNA, vol. 7, no. 9, 1988, pages 645-650.
D6: WO 96 32471 A (UNIV SHERBROOKE ;BEAUDOIN ADRIEN R (CA); SEVIGNY JEAN (CA)) 17 October 1996.
D7: EP-A-0 416 673 (CIGB) 13 March 1991.
D8: US-A-5 073 627 (CURTIS BENSON M ET AL) 17 December 1991.

3) Novelty (Articles 33.1 and 33.2 PCT)

The expression "the use of soluble CD39 polypeptide" shall be interpreted throughout this communication as "the use of soluble CD39 polypeptide for inhibiting platelet activation and recruitment in a mammal in need of such a treatment", except when specified otherwise.

Claims 1-11 relate to the use of a soluble CD39 polypeptide for inhibition of platelet activation and recruitment in a mammal in need thereof. Said polypeptide comprise the polypeptides constituted by amino acids [36-44 to 471-478] of the human CD39, fragments, variants and fusion polypeptides thereof.

The use of soluble CD39 polypeptides lacking the membrane spanning sequences for inhibiting platelet aggregation in a mammalian is known (see D2 claims 34-41). The spanning sequences correspond to amino acids [17-37 and 477-499] of the polypeptide of SEQ ID:2 (see figure 1 of the application). Therefore the compounds of claim 1(a) and 2 cannot be distinguished from the compounds used in the prior art for the same purpose. Hence, **claims 1 and 2** lack novelty.

The use of soluble CD39 fusion polypeptides of claims 3-5, 8 is not disclosed in the prior art. The polypeptides of claims 6(b,c), 7 embrace polypeptides constituted by amino acids [36-44 to 471-478] of the human CD39 which use is disclosed in the prior art (D2). For instance the polypeptide consisting of amino acids [25-464] of SEQ ID:27 is the polypeptide consisting of amino acids [38 to 476] of the human CD39 (SEQ ID:2) with one additional Alanine residue in position 25. Hence, **claims 3-5, 8** are novel whereas **claims 6-7** lack novelty.

The use of soluble CD39 polypeptide is known (D2). When specifications of the mode of production of the compound do not modify the nature or properties of the polypeptide, these specifications cannot bring novelty to the use of this particular compound. Therefore, **claim 9** lacks novelty.

Claims 10-11 relate to the use of the polypeptides encoded by the nucleic acid of SEQ ID:5 or SEQ ID:7 or variants of these sequences. Said polypeptides encode respectively the polypeptides of SEQ ID:6 and 8. Whereas, the use of the polypeptide of SEQ ID:6 and 8 could be regarded as novel, it is believed that a polypeptide constituted by amino acids [36-44 to 471-478] of the human CD39 could be encoded by variants of the nucleic acid of SEQ ID:5 and 7 (the polypeptide of SEQ ID:6 and 8 are respectively ~96% and ~91% identical to the polypeptide consisting of amino acids [38 to 476] of the human CD39). Therefore, **claims 10 and 11** lack novelty.

Claims 12-16 relate to different modes of administration of soluble CD39 polypeptide. D2

discloses a pharmaceutical composition of a soluble ecto-ATP diphosphorylase analogue in a pharmaceutically acceptable carrier suitable for intravenous injection (page 20 line 26-33 and page 28, line 13-19). Therefore, **claims 12, 15-16** lack novelty. Co-administration of the soluble CD39 polypeptide with antithrombic composition or aspirin is not disclosed for inhibiting platelet activation and recruitment, therefore **claims 13-14** are novel.

Claim 17-19 relates to the application of the use of soluble CD39 polypeptide to the treatment of specified diseases or conditions. D2 discloses such applications (page 30), particularly for thrombotic conditions, atherosclerosis and bypass surgery. Therefore, **claims 17-19** lack novelty.

Claim 20 relate to the use of soluble CD39 polypeptides for degrading nucleoside tri- and/or di- phosphates in a mammalian need thereof, which is merely the mechanism of action underlying the inhibition of platelet activation and recruitment. Moreover D2 discloses that the polypeptides used for inhibiting platelet activation and recruitment have ATP diphosphohydrolase activity (claim 34). Therefore, **claim 20** lacks novelty.

4) Inventive Step (Articles 33.1 and 33.3 PCT)

D2 which is considered to be the closest prior art, discloses the use of soluble polypeptides consisting of soluble CD39 polypeptides lacking the membrane spanning sequences (which is considered equivalent to amino acid [36-44 to 471-478] of polypeptide of SEQ ID:2) for inhibiting platelet activation and recruitment in a mammal in need of such a treatment. The application can partly be distinguished from this prior art as different soluble CD39 polypeptides are used, namely fusion proteins in which one or more amino acid residue were added to the N-terminus of the soluble CD39 polypeptides. This addition improve the expression level and/or stability of the CD39 polypeptide (page 11 line 21-31). Nevertheless, those effects are foreseen by D5 and appear to be both related to the production of the CD39 polypeptide in a recombinant cell rather than to the present invention which relate to later use of the CD39 polypeptide. Therefore, the solution proposed by the application (using fusion soluble CD39 polypeptides) to inhibit platelet activation and recruitment does not bring any unexpected effect (with regard to the biological activity) over the known use of a soluble CD39 polypeptide for inhibiting platelet activation and recruitment. Moreover, the use of fusion soluble CD39 polypeptide for inhibiting platelet activation and recruitment is strongly suggested in D1 (§ "discussion").

Co-administration with other antithrombic composition or aspirin is not regarded as inventive in the absence of a synergistic effect as it would be trivial for the men skilled in the art to combine two compositions know to achieve the same effect in order to achieve said effect.

Consequently, **claims 1-20** lack an inventive step.

5) Industrial applicability (Articles 33.1 and 33.4 PCT)

For the assessment of the present **claims 1-20** on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VII
Certain defects in the international application

6) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2 and D5 are not mentioned in the description, nor are these documents identified therein.

Re Item VIII
Certain observations on the international application

7) **Claim 1(a)** rely on reference to figure, which does not appears absolutely necessary (Rule 6.2(a) PCT). The applicant is invited to redraft the claim with a direct reference to SEQ ID NO:2.

8) Although **claims 3, 5, 6, 7, 9** have been drafted as separate independent claims, they appear to relate effectively to related subject-matter, e.g. particular embodiements of the subject-matter of claim 1. The aforementioned claims therefore lack conciseness. It would appear appropriate to file an amended set of claims defining the relevant subject-matter

in terms of a minimum number of independent claims in each category followed by dependent claims covering features which are merely optional (Rule 6.4 PCT).

PATENT COOPERATION TREATY

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	02.03.2001
Applicant's or agent's file reference P23,495 PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/23641	13/10/1999	16/10/1998

Applicant
IMMUNEX CORPORATION et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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Form PCT/IPEA/416 (July 1992)

23,495 PCT

CLAIMS

We claim:

1. A method for inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide having a structure X-Y wherein X is selected from the group consisting of an Ala residue and heterologous peptides capable of adopting a stable secondary structure and Y is selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; and

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity.

2. The method of claim 1 wherein Y is selected from the group consisting of:

(a) polypeptides having a sequence consisting of amino acids 38-476 or 39-476 of SEQ ID NO:2,

(b) variant polypeptides that are at least 70% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(c) variant polypeptides that are at least 80% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(d) variant polypeptides that are at least 90% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(e) variant polypeptides that are at least 95% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity;

(f) variant polypeptides that are at least 98% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity; and

(g) variant polypeptides that are at least 99% identical in amino acid sequence to amino acids 36 to 478 of SEQ ID NO:2 or to a fragment thereof, wherein said variant polypeptides have apyrase activity.

3. The method of claim 1 wherein X is a peptide fragment from the amino terminal portion of mature IL-2, CD39-L2, CD39-L3, or CD39-L4.

4. The method of claim 1 comprising administering a polypeptide having the structure A-B-Y wherein A is 0-20 amino acids from the amino terminal portion of mature IL-2 and B is a linker of 0-15 amino acids.

5. A method of inhibiting platelet activation and recruitment in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide selected from the group consisting of:

(a) SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, or amino acids 21-463 of SEQ ID NO:30; and

(b) fusion polypeptides comprising the polypeptides of (a), wherein said fusion polypeptides have apyrase activity.

6. The method of claim 5 wherein the soluble CD39 polypeptide has an amino acid sequence selected from the group consisting of SEQ ID NO: 6, amino acids 25-464 of SEQ ID NO:27, amino acids 25-474 of SEQ ID NO:28, amino acids 27-473 of SEQ ID NO:29, amino acids 21-476 of SEQ ID NO:3, amino acids 21-476 of SEQ ID NO:4, and amino acids 21-463 of SEQ ID NO:30.

7. The method of claim 6 wherein the soluble CD39 polypeptide has the sequence of amino acids 21-463 of SEQ ID NO: 30.

8. A method according to one of claims 1-7 wherein the soluble CD39 polypeptide has been produced by culturing a recombinant cell that encodes the soluble CD39 polypeptide under conditions permitting expression of the CD39 polypeptide, and recovering the expressed CD39 polypeptide.

9. The method of claim 8 wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:5; and

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO:5.

10. The method of claim 8 wherein the recombinant cell comprises a nucleic acid having a sequence selected from the group consisting of:

(a) SEQ ID NO:7; and

(b) DNA sequences which, due to degeneracy of the genetic code, encode the polypeptide encoded by SEQ ID NO.7.

11. The method of one of claims 1-10 wherein the soluble CD39 polypeptide is administered in a composition comprising a pharmaceutically acceptable carrier.

12. The method of one of claims 1-11 wherein the soluble CD39 polypeptide is administered in combination with at least one other antithrombotic or antiplatelet composition.

13. The method of claim 12 wherein the soluble CD39 polypeptide is administered in combination with aspirin.

14. The method of one of claims 1-13 wherein the soluble CD39 polypeptide is administered parenterally.

15. The method of claim 14 wherein the soluble CD39 polypeptide is administered intravenously.

16. The method of one of claims 1-15 wherein the mammal is suffering from unstable angina, myocardial infarction, stroke, coronary artery disease or injury, myocardial infarction, atherosclerosis, peripheral vascular occlusion, preeclampsia, embolism, a platelet-associated ischemic disorder including lung ischemia, coronary ischemia, and cerebral ischemia, a thrombotic disorder including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombosis and coagulopathy associated with exposure to a foreign or injured tissue surface, deep venous thrombosis (DVT), pulmonary embolism (PE), transient ischemic attack (TIAs), or another related condition where vascular occlusion is the common underlying feature.

17. The method of one of claims 1-15 wherein the soluble CD39 is administered to prevent thrombus formation or reformation, occlusion, reocclusion, stenosis, or restenosis of blood vessels, or stroke.

18. The method of one of claims 1-15 wherein the soluble CD39 is administered in conjunction with angioplasty, carotid endarterectomy, anastomosis of vascular graft, atherectomy, stent placement, placement of a chronic cardiovascular device such as an in-dwelling catheter or prosthetic valve or vessel, or bypass surgery.

19. A method for degrading nucleoside tri- and/or di- phosphates in a mammal in need of such treatment comprising administering an effective amount of a soluble CD39 polypeptide having a structure X-Y wherein X is selected from the group consisting of an Ala residue and heterologous peptides capable of adopting a stable secondary structure and Y is selected from the group consisting of:

(a) polypeptides having an amino acid sequence as set forth in (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478;

(b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; and

(c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity.

Immunoprecipitation of HUVEC detergent lysates with anti-CD39 mAb resulted in complete capture of cell-associated ADPase activity, suggesting that CD39 is the only ecto-ADPase on endothelial cells (Marcus et al., *J. Clin. Invest.* 99:1351, 1997). In the same study, COS cell transfectants expressing recombinant CD39 at the cell surface totally inhibited ADP-induced platelet aggregation. Thus, CD39 plays a prominent role in thromboregulation (*see also*, Gayle et al., *J. Clin. Invest.*, 101:1851, 1998; WO96/30532).

Excessive platelet activation (i.e., stimulation by an agonist) and recruitment, leading to platelet aggregation and vessel occlusion at sites of vascular injury in the coronary, carotid, and peripheral arteries, presents a major therapeutic challenge in cardiovascular medicine. Excessive platelet activation and recruitment is a contributing factor in clinical disorders including stroke, unstable angina, myocardial infarction, and restenosis following percutaneous coronary intervention including angioplasty, atherectomy, stent placement, and bypass surgery.

Glycoprotein IIb/IIIa antagonists, such as the monoclonal antibody marketed as ReoPro® (Centocor Inc.), are presently under development for the inhibition of platelet aggregation in patients undergoing percutaneous coronary intervention, and in patients with acute coronary syndromes such as unstable angina and myocardial infarction. The activation of glycoprotein IIb/IIIa receptors, however, is a late event in the cascade that leads to platelet aggregation.

There is a great need to identify additional therapeutic strategies and compositions for the pharmacological neutralization of platelet reactivity (activation, recruitment, aggregation). In particular, there is a need to identify compounds and compositions which target early portions of coagulation pathways such as the ADP-dependent activation and recruitment of platelets. There is, in fact, an urgent need to identify new strategies and compositions for the treatment of stroke, which is the third leading cause of death in the United States. In the case of stroke, an advantageous therapeutic agent will reduce intravascular thrombus burden and accompanying neurological defects without increasing intracerebral hemorrhage.

SUMMARY OF THE INVENTION

Soluble forms of CD39 having apyrase activity constitute a novel approach to the prevention and/or treatment of disease. The present invention provides soluble CD39 polypeptides and nucleic acids, compositions comprising a pharmaceutically acceptable carrier and a soluble CD39 polypeptide, and methods of making and using soluble CD39 polypeptides having apyrase activity. The effectiveness of soluble CD39 polypeptides have been demonstrated in vitro, ex vivo, and in vivo.

The invention is directed to soluble CD39 polypeptides selected from the group consisting of: (a) polypeptides having an amino acid sequence as set forth in Figure 1 (SEQ ID NO:2) wherein the amino terminus is selected from the group consisting of amino acids 36-44, and the carboxy terminus is selected from the group consisting of amino acids 471-478; (b) fragments of the polypeptides of (a) wherein said fragments have apyrase activity; (c) variants of the polypeptides of (a) or (b), wherein said variants have apyrase activity; and (d) fusion polypeptides comprising the polypeptides of (a), (b),